Nano-Check™ NT-proBNP Test

For in vitro Diagnostic Use

One–step immuno-chromatographic assay for the detection of NT-proBNP in human whole blood, serum, and plasma

1. Intended Use

The Nano-Check™ NT-proBNP Test is a rapid immunoassay for determination of NT-proBNP (N-terminal pro-Brain natriuretic peptide) in human whole blood, serum and plasma specimens at cutoff concentrations of 125 pg/mL for patients younger than 75 years and 450 pg/mL for patients 75 years and older, as an aid in the diagnosis of individuals suspected of having CHF (congestive heart failure). The test results should be interpreted by the cardiac specialist in conjunction with other clinical information such as patient's clinical symptoms and other test results to diagnose CHF.

2. Summary and Principle

The natriuretic peptides are a family of molecules consisting of several structurally-related hormones including arterial natriuretic peptide (ANP), B-type (or brain) natriuretic peptide (BNP), C-type natriuretic peptide (CNP), and dendroaspis natriuretic peptide (DNP). B-type natriuretic peptides are produced initially as a 134 amino acid pre-pro-peptide, which is cleaved into proBNP as 108 amino acids and this precursor molecule is stored in secretary granules in myocytes. Upon release, proBNP is cleaved by a protease known as furin into N-terminal (NT)-proBNP (a 76 amino acid, biologically-inert portion), and BNP (which is biologically active). In humans, NT-proBNP and BNP are found in largest concentration in the left ventricular (LV) myocardium but are also detectable in arterial tissue as well as in the myocardium of the right ventricle. A significant body of evidence has developed to demonstrate that NT-proBNP and BNP levels correlate with diagnosis, clinical status and prognosis in congestive heart failure, and may be useful for the longitudinal management of patients with CHF.

The Nano-Check™ NT-proBNP Test is an immuno-chromatography assay for quantitative determination of NT-proBNP in human whole blood, serum, and plasma specimen. The membrane strip contains a test line and a control line, printed with streptavidin for biotinylated NT-proBNP antibody and rabbit anti-goat IgG antibody for control line. A dye pad containing biotinylated NT-proBNP antibody and gold colloidal particles coupled with NT-proBNP antibody is placed at the end of the membrane. When a sample is applied into the sample well, the NT-proBNP molecules in the sample bind to both NT-proBNP specific dye coupled antibody and biotinylated antibody. These immune complexes move along the nitrocellulose membrane through the test lines and bind to streptavidin immobilized on the test lines.

If the concentration of markers NT-proBNP in the sample is above the detection limit level (30 pg/mL), red bands appear at the test line and the control line. If the concentration of NT-proBNP in the sample is lower than the detection limit level, only the colored control line can be seen in the test window. This colored control band must always appear at the control line position (Con) for valid test results. A test result is not valid if the colored control line does not appear in the test window. To measure the concentration of analyte, the tested device should be read by Nano-Checker™ 710 reader. The reader can analyze color intensity of the test line and convert it to concentration of the analyte in the specimen by the predetermined equation.

3. Reagent

The Nano-Check™ NT-proBNP Test contains all the reagents necessary for the detection of NT-proBNP in human whole blood, serum, and plasma. The device contains a membrane strip coated with streptavidin on the test line, and dye pad infused with biotinylated monoclonal mouse anti- NT-proBNP antibody and gold colloidal particles coupled with anti- NT-proBNP antibodies. Stabilizer containing 0.05% sodium azide, 5% trehalose and other chemicals including sodium phosphate for buffer capacity are deposited on the dye pad as dried form.

4. Materials

Provided

- 20 Nano-Check™ NT-proBNP Test devices containing membrane strip in a sealed pouch with desiccant
- 20 Disposable droppers
- Instruction for use

Required but not provided

- Whole blood, Serum or Plasma Collection Container
- Positive and negative quality control materials (Nano-Check Cardiac Control: require separate purchase)
- Timer
- Nano-Checker™ 710 Reader

5. Storage and stability

The test kit should be stored at 2°C - 30°C in the original sealed pouch for the duration of shelf life.

6. Precautions

- For *in vitro* diagnostic and professional use only.
- Do not use hemolyzed specimens as hemolysis affect test results
- Handle all specimens as potentially infectious. Proper handling and disposal methods should be established.
- To avoid cross contamination, use a fresh transfer pipette for each clinical sample tested.
- Do not use test kit if the pouch is damaged or improperly sealed.
- Do not use test kit beyond expiration date.

7. Specimen collection and preparation

- This test can be used for whole blood, plasma, and serum samples. If serum samples are to be used, collect the blood in a tube without anticoagulant and allow clotting for at least 25 minutes before centrifugation. Whole blood or plasma samples using heparin or EDTA as the anticoagulant can be used for testing with this product. Other blood anticoagulants have not been evaluated. Each laboratory should determine the

acceptability of its own blood collection tubes and serum separation products. Variation in these products may exist between manufacturers and, at times from lot-to-lot.

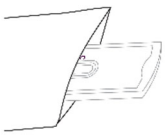
- **Do not use whole blood which was not treated with anticoagulant**
- The samples should be collected under standard laboratory conditions.
- Optimal results were obtained when patient samples were tested immediately after collection. Whole blood samples should be used within 4 hours after collection. Plasma or serum samples may be refrigerated for 24 hours at 2-8°C. If testing cannot be performed within 24 hours, or for shipment of samples, freeze at -20°C or below.
- Sodium azide can be added as a preservative up to 0.1% without affecting the test results.
- Refrigerated or frozen serum or plasma specimen should reach room temperature and be homogeneous prior to testing

8. Test procedure and protocol

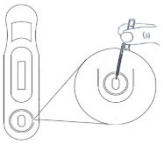
a) Collect specimen according to instructions in "Specimen Collection".

b) Test device and sample should be brought to room temperature prior to testing.

c) Remove the test device from the sealed pouch immediately before use. Label the device with patient or control identification.



d) Using sample transfer pipette, deliver dropper contents (80 μL) of sample into the sample well.



e) Read the results at 15 minutes using the Nano-Checker™ 710 Reader. Follow the procedure in the user manual of Nano-Checker™ 710 Reader.

9. Interpretation of results

The signal intensities of test lines are analyzed by Nano-Checker™ 710 Reader and reported as concentrations of analyte in the tested specimen. When the test is valid and the measured result is in the range of suggested reference value, the result can be interpreted as a negative. If the measured result is above the suggested reference range, the result can be interpreted as a positive. **The results from this or any other diagnostic test should be used and interpreted only in the context of the overall clinical picture**

10. Expected values

The cutoff values of the Nano-Check™ NT-proBNP Test were estimated by comparison to the Roche's Elecsys® pro-BNP Immunoassay. The cutoff level of NT-proBNP is 125 pg/mL for patients younger than 75 years and 450 pg/mL for patients 75 years and older. However, each laboratory should establish its own reference range. It has been noted that the cutoff levels are different if a quantitative assay system other than Roche's Elecsys® pro-BNP Immunoassay is used.

Limitation: *Patients taking more than 30 μg/day of biotin may have falsely negative results and should not use this test, unless it is conformed that the patient is not taking more than 30 μg/day of biotin.*

11. Quality Control

The presence of a reddish colored band in the Control area of the window acts as an internal control to ensure that an adequate volume of sample has been added. In the absence of this Control band, the test is invalid and must be repeated. Good laboratory practice recommends the use of control materials to ensure proper kit performance. Quality control specimens are available from commercial sources and should be assayed using the same procedures followed when running patient samples. Controls should minimally be run before using each new lot or shipment of Nano-Check™ NT-proBNP Test, at regular intervals afterwards and any time the validity of the test results are questioned.

For the calibration of quantitative reader, a calibration card for Nano-Checker™ 710 reader is supplied with the reader. Before measuring tested device, the reader should be calibrated with the provided calibration card. If the reading value of calibration card is out of described range, it should be recalibrated. Nano-Check™ Cardiac Controls can be used for positive and negative reference. According to the regulation of each operation lab, the device should be validated periodically using NT-proBNP control solution as reference.

12. Performance Characteristics

1. Precision Test

Total imprecision of the Nano-Check™ NT-proBNP test with Nano-Checker™ 710 Reader was determined in study using plasma based in-house control materials. Specimens, negative human plasma pools spiked with NT-proBNP concentration at each level, were tested over 20 times for 10 days. The within and total standard deviation were calculated by the analysis of variance method.

NT-proBNP (pg/mL)	Within run		Total Precision	
	CV%	95% CI	CV%	95% CI
300	11.9	10.8~13.3	12.7	11.6~14.5
600	11.1	10.0 ~12.3	11.4	10.4 ~12.8
1500	10.4	9.5~11.6	11.1	10.1 ~12.6
3000	11.1	10.0 ~12.3	11.5	10.5 ~13.0

2. Recovery Studies

Recovery studies were performed by testing the calibrator controls which were heparinized plasma containing NT-proBNP antigens. The calibrators were made by serially diluting with normal human plasma to yield different dilution concentrations. Each diluted sample was tested using Nano-Check™ NT-proBNP test in 10 replicates. The data shown in the table below demonstrated recovery rate between observed results and expected results at each concentration of NT-proBNP.

Analyte	Expected Concentration (pg/mL)	Determined Concentration	Agreement Expected values (%)	Recovery Rate (%)
NT-proBNP	150	151.3	101	98.2
	300	298.0	99	
	600	529.3	88	
	1500	1465.8	98	
	3000	3056.8	102	
	6000	6043.0	101	

3. *Analytical Sensitivity*

The analytical sensitivity was determined according to the standard CLSI EP17-A. Eighty (80) replicates of each NT-proBNP depleted human plasma (blank sample) were tested for LoB determination. Blank sample was spiked with recombinant human NT-proBNP protein at low positive concentrations of 1× LoB, 1.5× LoB, 2× LoB, 2.5× LoB or 3× LoB and 10 replications of each low positive sample were tested for tentative LoD determination. The LoD was verified by testing 60 replications of LoD verification sample. In this study, LoB was determined to 12.9 pg/mL. The LoD was determined to 27.0 pg/mL and LoQ was verified to 30 pg/mL.

4. *Interference study*

Potentially interfering substances were spiked into normal plasma containing recombinant NT-proBNP concentration of 0 and 400 pg/mL. The substances at the following level do not cause a bias of over 15% with the test at the concentration of NT-proBNP.

Substances	Concentration
Bilirubin	0.1 mg/mL
Hemoglobin	1 mg/mL
Triglycerides	10 mg/mL
Cholesterol	5 mg/mL
BNP	10 µg/mL
ANP	10 µg/mL
CNP	10 µg/mL
Rheumatoid factor	993 IU/mL
Biotin, Vitamin B7	300 ng/mL

5 *Cross- Reactivity*

The cross reactivity of the Nano-Check™ NT-proBNP test was evaluated by spiking potential cross-reacting drug compound to the normal human plasma at the concentration of 10 µg/mL. There was no significant interference with the analyte, nor was there any assay cross-reactivity.

Acetaminophen	Dopamine	Oxytetracycline
Acetylsalicylic acid	Erythromycin	PCP
Allopurinol	Fluoxetine	Phenobarbital
Ampicillin	Furosemide	Phenytoin
Ascorbic acid	Hydrochlorothiazide	Probenecid
Atenolol	Hydrocodone	Procainamide
Caffeine	Ibuprofen	Propranolol
Captopril	Indomethacin	Quinidine
Chloramphenicol	Metoprolol	Sulfamethoxazole
Cocaine	Morphine	Theophyline
Digoxin	Nicotine	Verapamil
Diltiazem	Nitrofurantoin	Warfarin
Dipyridamole	Nitroglycerin	

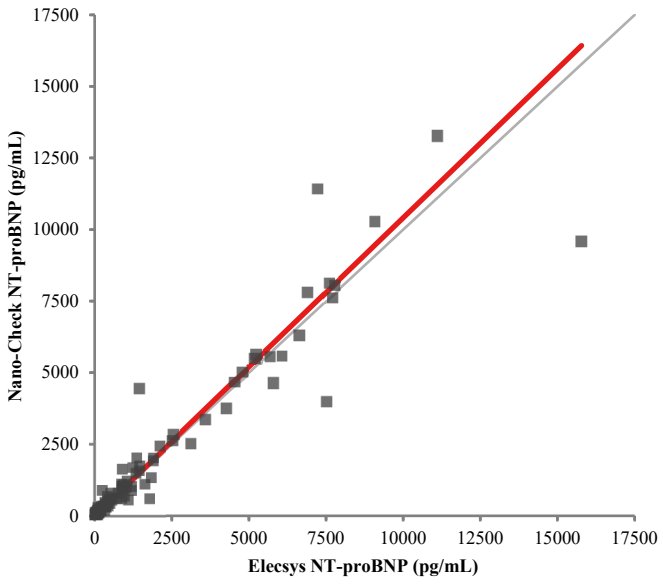
6. *Matrix comparison study*

To perform matrix comparison study between plasma and whole blood in Nano-Check™ NT-proBNP test, 10 different levels of analyte concentrations ranging from 40pg/mL to 12000 pg/mL were prepared by spiking analyte molecules into normal whole blood pool collected from 10 different healthy volunteers. Corresponding plasma specimens were prepared from each level of whole blood specimens by centrifugation. Each level of whole blood or plasma specimens were run on the same lot of Nano-Check™ NT-proBNP device in 4 replicates. The concentrations were measured using the analysis programs for plasma test on Nano-Checker™ 710 reader.
Regression and correlation were analyzed by comparing average of each specimen (heparinized whole blood, EDTA treated plasma, EDTA treated whole blood, and serum) result with average of heparinized plasma specimen. This study shows that the regression slope falls within the 0.9~1.1 range from every comparison and the correlation coefficient is >0.95 from every comparison.

7. *Method Comparison Study*

The method of comparison study was conducted using 127 samples. Clinical samples were collected from patients suspected of having CHF (congestive heart failure) and healthy volunteer. The concentration of NT-proBNP measured on Nano-Check™ NT-proBNP was plotted versus that on Elecsys® NT-proBNP shown in the figure below. The data was analyzed using the Passing-Bablok regression method and Spearman's ranked correlation method (ρ) and is summarized in the following table.

Ranges of Observation (pg/mL)	Slope	Correlation Coefficient
30 – 15,000	0.9238	0.965



Comparison data were further analyzed for concordance at the NT-proBNP cut-off level of 125 pg/mL for patient younger than 75 years and 450 pg/mL for patient older than 75 years, both of which were determined by a ROC analysis. The Nano -Check™ NT-proBNP Test showed 96.3% PPA, 91.3% NPA, and 94.5 OPA for patient younger than 75 years as well as 98.2% PPA, 94.4% NPA, and 96.1 OPA for patient older than 75 years.

PPA, NPA, and OPA at cut-off level of 125 pg/mL

Nano-Check™ NT-proBNP Test	Elecsys® NT-proBNP		Total	Performance (95% CI)
	< 125 pg/mL	≥ 125 pg/mL		
< 125 pg/mL	42	3	45	NPA*: 91.3% (79.7-96.6%)
≥ 125 pg/mL	4	78	82	PPA*: 96.3% (89.7-98.7%)
Total	46	81	127	OPA*: 94.5%

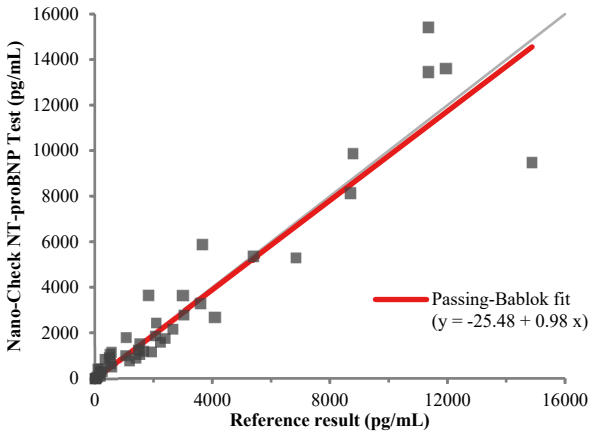
*NPA: Negative percent agreement; PPA: Positive percent agreement; OPA: Overall percent agreement

PPA, NPA, and OPA at cut-off level of 450 pg/mL

Nano-Check™ NT-proBNP Test	Elecsys® NT-proBNP		Total	Performance (95% CI)
	< 450 pg/mL	≥ 450 pg/mL		
< 450 pg/mL	68	1	69	NPA: 94.4% (86.6-97.8%)
≥ 450 pg/mL	4	54	58	PPA: 98.2% (90.4-99.7%)
Total	72	55	127	OPA: 96.1%

Additionally, a further method of comparison study was conducted using 55 clinical samples treated with heparin or EDTA as an anticoagulant. The concentration of NT-proBNP measured on Nano-Check™ NT-proBNP was plotted versus that on Simens Stratus CS shown in the figure below. The data was analyzed using the Passing-Bablok regression method and Spearman's ranked correlation method (ρ).

Ranges of Observation (pg/mL)	Slope	Correlation Coefficient
30 – 15,000	0.98	0.977



13. Reference

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12. Bibbins-Domingo K, Gupta R, Na B, et al. N-terminal fragment of the prohormone brain-type natriuretic peptide (NT-proBNP), cardiovascular events, and mortality in patients with stable coronary heart disease. JAMA 2007 Jan 10;297:169–76.

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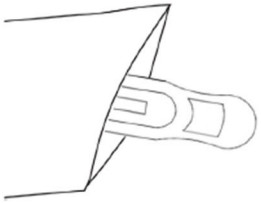
Quick Reference Instruction for Nano-Check™ NT-proBNP with Nano-Checker 710 Analyzer

Read the complete test procedure, including recommended QC before performing the test. Refer to the IFU for complete information about the test. Ensure ALL components are at room temperature when running the test.

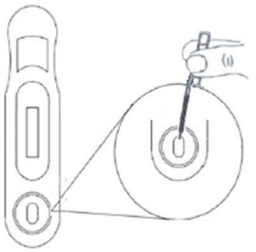
Sample preparation

① Collect whole blood, plasma, or serum specimen. Both the test cassette and sample should be brought to room temperature prior to testing.

② Remove the test cassette from the sealed pouch immediately before use.



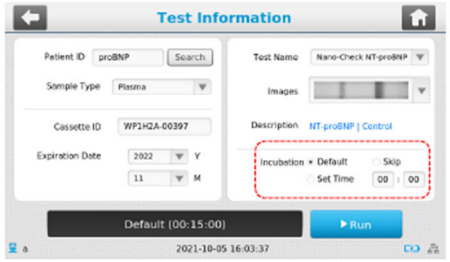
③ Deliver 80 µL of whole blood or plasma or serum sample into the sample well.



Using Nano-Checker 710 Analyzer to read the cassette

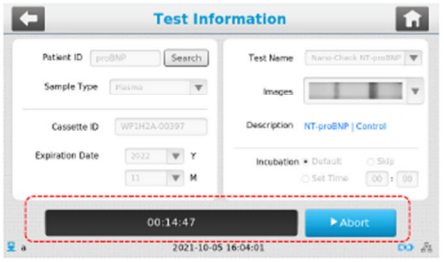
[Default Mode]

※ Set "Default" Mode

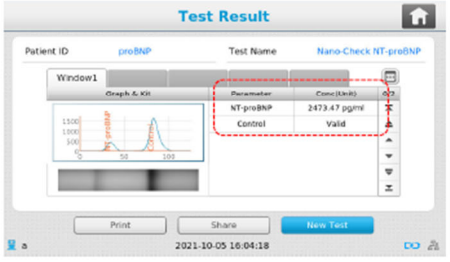


④ Insert the cassette immediately and press Run.

⑤ 15mins of incubation will automatically start.



⑥ Result will appear on screen in 15mins.



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